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Lateral Interactions among Phosphatidylcholine and Phosphatidylethanolamine Head Groups in Phospholipid Monolayers and Bilayers[†]

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ABSTRACT: We develop theory for the lateral interactions among the zwitterionic head groups of phospholipids in monolayers and bilayers, particularly phosphatidylcholine (PC) and phosphatidylethanolamine (PE). With the P^- end of the head group anchored at the water/hydrocarbon interface, a balance of two effects dictates the angle that the P^-–N^+ dipole makes with respect to the plane of the bilayer: N^+ is driven toward water due to the (Born) electrostatic free energy, but the hydrophobic effect drives the methyl and methylene groups around the N^+ charge toward the hydrocarbon. The only adjustable parameter of the model is the average fluctuation of the oil/water interface or, alternatively, the dielectric constant of the hydrocarbon phase. The model predicts that at 5 °C the head group dipole should lie largely in the bilayer plane, in accord with X-ray, neutron diffraction, and NMR studies. The theory makes the novel prediction that the N^+ end of the dipole becomes increasingly submerged in hydrocarbon with increasing temperature, leading to strongly enhanced lateral repulsion between PC head groups. This prediction is in good agreement with second and third virial coefficients of monolayer lateral pressures, and with the temperature dependence of the former. The theoretical model is consistent with head group fluctuations measured by neutron diffraction of PC and PE bilayers. Because PE has a smaller hydrophobic cluster near N^+, its lateral repulsion should be much smaller and less temperature dependent than for PC, also in agreement with equation-of-state measurements. This suggests why at high density PE monolayers have higher melting temperatures than PC monolayers and more propensity for reversed curvature.

For some time there have been two paradoxical observations regarding interactions among the principal zwitterionic phospholipids. (i) Structural studies (X-ray diffraction, neutron diffraction, ^{31}P NMR) have shown similar packing of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) head groups in bilayer membranes, yet their physical properties are very different: phase transition temperatures

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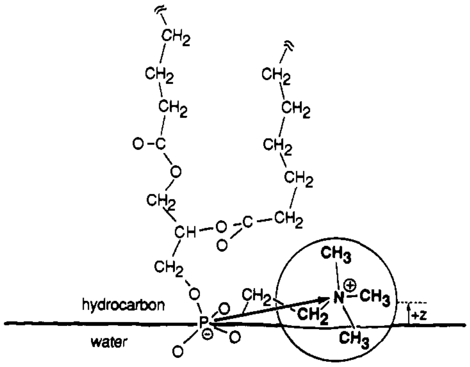

FIGURE 1: Schematic conformation of phosphatidylcholine at the hydrocarbon/water interface. The dihedral angles in the PN link are denoted, successively, α_3, α_4, α_5, and α_6.

differ, PE membranes fuse but PC membranes generally do not, PE readily forms inverted hexagonal micelles whereas PC does not, etc. It is common to assume that the differences in properties are due to hydrogen-bonding interactions at the N^+ end of the PE head group, which are not possible in PC. (ii) The structural observations show that both PC and PE head groups lie almost completely in the plane of the bilayer, which would imply a small net intermolecular attraction (Stigter & Dill, 1988), yet studies on phospholipid monolayers show relatively strong, temperature-dependent repulsions among PC head groups (Taylor et al., 1976).

We have recently shown (Stigter & Dill, 1988) through analysis of the second and third two-dimensional virial coefficients, B_2 and B_3, respectively, from the extensive lateral pressure/area isotherm measurements of Taylor et al. (1976), that the latter paradox is resolved by recognizing the importance of the very small out-of-plane component of the head group dipole. The principal conclusion of that work is that the only interaction which explains the strong repulsions arises when the N^+ end of the head group is oriented "backwards" into the hydrocarbon environment, rather than into the water phase. This is a consequence of the dependence of the potential on the inverse square of the dielectric constant: the interaction of head groups directed into the hydrocarbon region is about 3 orders of magnitude larger than when the head groups are aligned out of plane into the water and accounts for the B_2 and approximately for the B_3 values derived from the experiments. The net displacement of the N^+ end of the phospholipid into the hydrocarbon that is required to account for these large repulsive forces is only of the order of 1 Å, well within the experimental errors of the structural methods which show that the head group lies "essentially" in plane. Thus the equation-of-state experiments appear to be much more sensitive to head group orientation than the structural diffraction methods. Conversely, theoretical models of head group interactions will be very sensitive to small changes in orientation. In this paper we develop a molecular model that accounts for the interactions between PC and between PE head groups, and we discuss applications of the model to various membrane properties.

ENERGETICS OF PC HEAD GROUP AT HEPTANE/WATER INTERFACE

In the present work, we consider the driving force that, at equilibrium, causes the N^+ end of the PC head group to be displaced into the hydrocarbon region. In the model presented here, the P^- end of the head group is assumed to be fixed at the water/hydrocarbon interface [see Stigter and Dill (1988) for discussion]. The P^-–N^+ dipole is taken to be a rigid rod that pivots around the P^-, so that the head group orientation is described by a single degree of freedom, the vertical displacement of the N^+ charge from the interface into the oil phase, $z = l \sin\theta = t \sin\phi$ (see Figures 1 and 2). The equilibrium position of the N^+ is determined by a balance of two forces: on the one hand, the N^+ charge will be attracted to water due to the (Born) electrostatic free energy; on the other hand, the CH_2 and CH_3 groups attached to the N^+ will be attracted to the oil phase due to the hydrophobic effect. We note here the caveat that certain predictions of the theory below will be found to be very sensitive to details of the model, undoubtedly reflecting the physical reality. Nevertheless, this implies that there are important uncertainties, including those due to fluctuations of the interface whose effects are difficult to treat exactly. We now proceed with details of the contributions to the orientational free energy of the PC head group.

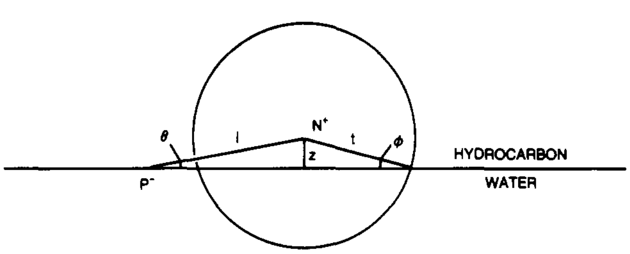

FIGURE 2: Model of PC head group. PN dipole of length $l = 4.5$ Å with hydrophobic sphere of radius $t = 3.5$ Å around N^+ charge at distance z into hydrocarbon phase.

Hydrophobic Free Energy. The N^+ charge is buried at the center of a hydrophobic sphere of methylene and methyl groups at the distal end of the head group. The van der Waals radius of the $N^+(CH_3)_3$ ion is $t = 3.5$ Å, and its center is $l = 4.5$ Å from the P^- charge (see Figure 2). We assume that the hydrophobic part of the free energy, F_h, changes in proportion to the contact area between sphere and water, given by

$$\text{contact area} = \int_{\pi/2}^{\phi} 2\pi t^2 \cos\phi' \, d\phi' = 2\pi t^2(1 - z/t) \qquad (1)$$

since $\sin\phi = z/t$. Thus we have

$$\begin{aligned} F_h &= 0 && z > t \\ F_h &= c_1(1 - z/t) && -t < z < t \qquad (2) \\ F_h &= 2c_1 && z < -t \end{aligned}$$

where $2c_1$ is the increase of F_h when the sphere is moved from heptane into water.

The quantity c_1 is derived from the expression of Gill and Wadsö (1976) for the free energy of transfer of hydrocarbons from oil into water:

$$2c_1 = T\frac{6.4 + 1.85n_H}{298} + T(0.033n_H)\left(-\ln\frac{T}{298} + \frac{T^*}{298} - \frac{T^*}{T}\right) \qquad (3)$$

where T is the absolute temperature and $n_H = 13$ is the number of H atoms surrounding the N^+ charge. Whereas for hydrocarbons $T^* \cong 295$ K, measurements by Bergström and Olofsson (1975) show that T^* is considerably higher for amines; e.g., $T^* \cong 403$ K for triethylamine and $T^* \cong 393$ K for dipropylamine, the compounds closest to the choline group for which data have been reported. We use $T^* = 400$ K in eq 3.

Electrical Free Energy. We now consider the electrical free energy, F_e, of the charged sphere at the N^+ end of the head group, at the interface between heptane with dielectric constant ϵ_h and water with dielectric constant ϵ_w. As in the treatment of the Born energy of ions [see, e.g., Ehrenson (1987)], F_e is

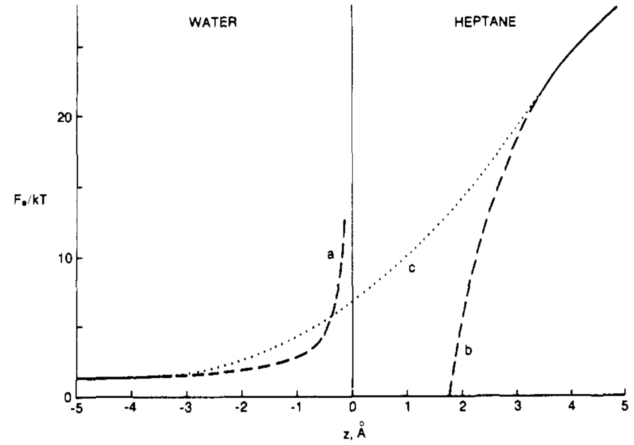

FIGURE 3: Electrostatic free energy F_e of hydrophobic sphere with radius t = 3.5 Å and charge e in center at distance z from heptane/water interface. Solid curves from eq 5 and 4, continued as dashed curves a and b. Dotted curve c from eq 6–10.

evaluated as the work to charge the sphere, that is, $F_e = \int_{\lambda=0}^{1} \psi(\lambda e)\, d(\lambda e)$, where $\psi(\lambda e)$ is the surface potential of the sphere with partial charge λe. In bulk heptane we have $\psi(\lambda e) = \lambda e/4\pi\epsilon_0\epsilon_h t$, and hence, $F_e = e^2/8\pi\epsilon_0\epsilon_h t$; ϵ_0 is the permittivity of free space. In bulk water, $F_e = e^2/8\pi\epsilon_0\epsilon_w t$.

When the sphere approaches the interface, its free energy gradually changes because the electrostatic field around it must satisfy the boundary conditions at the interface. In the case of a point charge, this free energy is computed by using the image method [see, e.g., Smythe (1968)], in which the dielectric boundary is replaced by the field of an imaginary charge at the mirror point at distance $2z$ from the point charge across the interface. As a result, for a point charge e in water, the free energy near the interface is larger than in the bulk, due to repulsion, by an amount $(e^2/16\pi\epsilon_0\epsilon_w z)(\epsilon_w - \epsilon_h)/(\epsilon_w + \epsilon_h)$, while in heptane the free energy near the interface is lower than in the bulk, due to attraction, by an amount $(e^2/16\pi\epsilon_0\epsilon_h z)(\epsilon_w - \epsilon_h)/(\epsilon_w + \epsilon_h)$. Thus, provided the charged hydrophobic sphere does not contact the interface, then in heptane

$$F_e = \frac{e^2}{8\pi\epsilon_0\epsilon_h t} - \frac{e^2}{16\pi\epsilon_0\epsilon_h z}\,\frac{\epsilon_w - \epsilon_h}{\epsilon_w + \epsilon_h} \qquad z > t \qquad (4)$$

and in water

$$F_e = \frac{e^2}{8\pi\epsilon_0\epsilon_w t} + \frac{e^2}{16\pi\epsilon_0\epsilon_w z}\,\frac{\epsilon_w - \epsilon_h}{\epsilon_w + \epsilon_h} \qquad z < -t \qquad (5)$$

In Figure 3 we have plotted the dimensionless quantity F_e/kT vs z, for t = 3.5 Å, with ϵ_h = 1.923 for n-heptane and ϵ_w = 78.54 for water at 25 °C. The solid lines given by eq 5 and 4 are continued as dashed lines a and b in the region $-t < z < t$. In the latter region, where the charged sphere crosses the planar dielectric boundary, eq 4 and 5 are not valid, and the exact solution for the electrostatic potential field, or for F_e, is now known. Therefore, we proceed with an approximation.

The electrical free energy F_e must be a continuous function of the position of the sphere. If we also assume continuity of dF_e/dz at $z = \pm t$, then we can interpolate the function through the interface using the lowest order polynomial expansion (curve c, Figure 3):

$$F_e = d_0 + d_1\left(\frac{z}{t}\right) + d_2\left(\frac{z}{t}\right)^2 + d_3\left(\frac{z}{t}\right)^3 \qquad -t < z < t \qquad (6)$$

with

$$d_0 = \frac{e^2}{16\pi\epsilon_0 t}\left[\frac{1}{\epsilon_w} + \frac{1}{\epsilon_h} + \frac{3}{4}\left(\frac{1}{\epsilon_w} - \frac{1}{\epsilon_h}\right)\frac{\epsilon_w - \epsilon_h}{\epsilon_w + \epsilon_h}\right] \qquad (7)$$

$$d_1 = \frac{e^2}{16\pi\epsilon_0 t}\left[\frac{-3}{2\epsilon_w} + \frac{3}{2\epsilon_h} - \left(\frac{1}{\epsilon_w} + \frac{1}{\epsilon_h}\right)\frac{\epsilon_w - \epsilon_h}{\epsilon_w + \epsilon_h}\right] \qquad (8)$$

$$d_2 = \frac{e^2}{64\pi\epsilon_0 t}\left(\frac{-1}{\epsilon_w} + \frac{1}{\epsilon_h}\right)\frac{\epsilon_w - \epsilon_h}{\epsilon_w + \epsilon_h} \qquad (9)$$

$$d_3 = 0 \qquad (10)$$

The quadratic interpolation function, the dotted curve c in Figure 3, gives over part of the range a higher free energy than that of curve a, which represents a point charge on the water side of the interface. A better approximation might consider the electric field inside the sphere with radius t to be constant. That case, as in the treatment of the Born energy of ions, is equivalent to the charge being uniformly distributed over the spherical surface, and we should expect that the electrostatic free energy would increase linearly with the sphere/water contact area, i.e., linearly with z/t as in eq 1 for the hydrophobic contribution. Such an approach would suggest that the influence of the oil phase on the electrical energy of the charged sphere could be higher than the image effect on the point charge considered here. Alternatively, a better approximation for the electrical free energy might be based on the work by Raudino and Mauzerall (1986) on dielectric properties of zwitterionic bilayers, or perhaps on recent work on the Born energy (Ehrenson, 1987). In our opinion, such refinements are unwarranted at the present stage of simplicity of other aspects of the model.

The treatment above applies when the oil/water interface is sharp and stationary. An important feature of real systems, however, is that the interfacial position will fluctuate locally in time. These thermal fluctuations will contribute significantly to $F_e(z)$. We represent these fluctuations as a Fourier series of plane waves. The wavelengths relevant to the present problem range from molecular size to the range of the electrostatic field of the N^+ charge. As shown in the Appendix, application of the equipartition theorem leads to a prediction that the average fluctuations of the local position of the oil/water interface, Δz, are around 2.5 or 3 Å. This value is confirmed by low-angle X-ray scattering experiments (Robinson, 1987; Weiss et al., 1987). A rigorous accounting for such fluctuations in the treatment of F_e is difficult. The main effect is that the central part of the F_e vs z curve, between $z = -t$ and $z = t$ = 3.5 Å, is "stretched out" over the region between $-t - \Delta z$ and $t + \Delta z$ (see Figure 3). This effect of interfacial fluctuations is approximately taken into account by substituting $t + \Delta z$ for t in eq 6; the coefficients in eq 7–10 remain unchanged.

The influence of interfacial fluctuations on F_h is expected to be relatively small because hydrophobic effects are expected to be short ranged. Fluctuations of the water/sphere contact area corresponding to Δz = 3 Å would be about $5kT$ in F_h and, hence, are very unlikely. It is consistent with our calculations to attach the z coordinate to the heptane/water/sphere boundary line, smoothed to a circle by averaging local fluctuations. In this case z = 0 when N^+ is in the plane through this circle, irrespective of close or distant fluctuations of the interface. Moreover, the time average of the circle is the time-average position of the oil/water interface. In this way it is clear that, because of the long-range nature of electrostatic fields, F_e is influenced by fluctuations that leave F_h unchanged. It is difficult to estimate to what degree the interfacial fluctuations cancel in the interaction between head

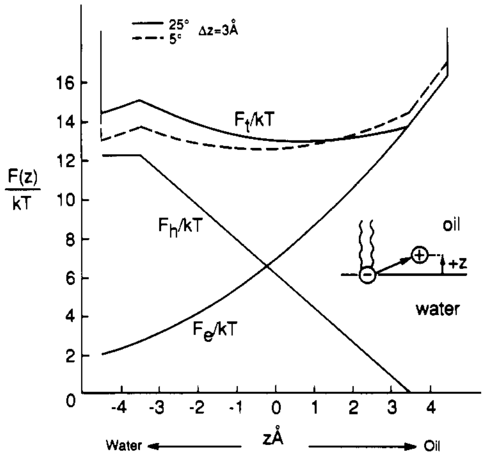

FIGURE 4: Free energy functions of charged hydrophobic sphere with radius $t = 3.5$ Å and charge e in center at distance z from heptane/water interface. Hydrophobic free energy F_h from eq 2 and 3. Electrostatic free energy F_e for average interfacial fluctuations $\Delta z = 3$ Å. Total free energy F_t from eq 11 and 12. Solid curves at 25 °C; dashed curve at 5 °C.

groups, that is, in the calculation of B_2. This adds an uncertainty to the calculated positions of the N^+ charge that is not easily assessed.

Total Free Energy of Head Group Orientation. We combine the hydrophobic and the electrostatic contributions to give the total free energy of the charged sphere

$$F_t(z) = F_h(z) + F_e(z) \tag{11}$$

Since we assume the P^- charge to be at the interface, the sphere cannot be displaced further from the interface than the length of the P–N vector, $l = 4.5$ Å; that is, $F_t = \infty$ for $z < -l$ and $z > l$. The central part of the curve is obtained from

$$F_t(z) = c_1\left(1 - \frac{z}{t}\right) + d_0 + d_1\left(\frac{z}{t + \Delta z}\right) + d_2\left(\frac{z}{t + \Delta z}\right)^2 \qquad -t < z < t \tag{12}$$

where the coefficients are given by eq 3 and 7–9.

The total free energy F_t is used to determine the average position of N^+, its normal fluctuations, and, in the next section, the second virial coefficient, B_2, of fluctuating head groups. The average value of z is obtained from

$$z_{av} = \frac{\int_{-l}^{l} z e^{-F_t(z)/kT}\,dz}{\int_{-l}^{l} e^{-F_t(z)/kT}\,dz} \tag{13}$$

and the mean square fluctuation of the N^+ position is given by

$$\mu^2 = \frac{\int_{-l}^{l} (z - z_{av})^2 e^{-F_t(z)/kT}\,dz}{\int_{-l}^{l} e^{-F_t(z)/kT}\,dz} \tag{14}$$

The free energy of the PC head group orientation and its electrostatic and hydrophobic contributions are shown in Figure 4. In the figure, F_h is from eq 2 and, in the central part, F_e is from eq 6 with the bulk values of ϵ_w and ϵ_h for water and heptane, respectively. F_t is from eq 11 and 12, with $\Delta z = 3$ Å. These free energies are shown as solid curves for 25 °C and as a dashed curve for F_t at 5 °C. From 5 to 25 °C the position of the minimum in the F_t curve changes from $z_{min} = -0.39$ to 0.86 Å. Due to the asymmetry of $F_t(z)$ the averages of z, obtained from eq 13 with $l = 4.5$ Å, are lower than z_{min} and range from $z_{av} = -0.55$ to 0.50 Å. The root mean square (rms) fluctuations, from eq 14, are found to be nearly constant, $\mu \cong 1.9$ Å between 5 and 25 °C. This compares with $\mu = 2.1 \pm 0.4$ Å for the distal CD_2 fragment in the head group measured by neutron diffraction of PC bilayers at 20 °C in the $L_{\beta'}$ phase, and with $\mu = 2.5 \pm 0.4$ Å at 70 °C in the L_α phase (Büldt et al., 1979).

The main conclusion from these calculations is that, at low temperature (5 °C), the PC head group is oriented on average with its N^+ end directed slightly into the water phase, but because the hydrophobic interaction strengthens with temperature and the electrostatic effects weaken with temperature, the hydrophobic N^+ end of the dipole is directed increasingly toward the hydrocarbon phase with increasing temperature.

SECOND VIRIAL COEFFICIENT

The orientation of the head group dipoles contributes to strong lateral repulsion among phospholipids (Stigter & Dill, 1988). This lateral repulsion, as determined from the second virial coefficient, B_2, of the lateral pressure of phosphatidylcholine at the heptane/water interface (Taylor et al., 1976), is comprised of steric and dipolar components. We have previously shown that the steric component can be suitably modeled by head groups as hard disks; shape anisotropy of the elongated head groups contributes negligibly. Therefore, we model the PC head group as a hard disk with radius $a = 4$ Å, and with dipole charges on the central axis. Consistent with the model for F_t above, we assume a charge $-e$ at the interface at $z = 0$ and the charge $+e$ at a distance z into the hydrocarbon.

Let r be the lateral distance between two head groups and let the vertical positions of their $+e$ charges be z_1 and z_2, respectively. Then B_2 depends on the pair potential $U_{12}(r,z_1,z_2)$ between the two dipoles as they fluctuate in the fields $F_t(z_1)$ and $F_t(z_2)$ given by eq 11. The potential U_{12} is evaluated as a sum of Coulomb terms, and B_2 is computed by using the following integral, derived from eq 20 and 24 of Stigter and Dill (1988) with the relation $z_i = l \sin \theta_i$:

$$B_2 = 2\pi a^2 - \frac{\pi \int_{2a}^{\infty} \int_{-l}^{l} \int_{-l}^{l} e^{-F_t(z_1)/kT} e^{-F_t(z_2)/kT} \left(e^{-U_{12}(r,z_1,z_2)/kT} - 1\right) dz_1\,dz_2\,dr}{\left(\int_{-l}^{l} e^{-F_t(z)/kT}\,dz\right)^2} \tag{15}$$

The term $2\pi a^2$ in eq 15 is the hard core contribution to B_2, for which $U_{12} = \infty$ for head group overlap, $r < 2a$.

The theoretical predictions for B_2 are shown in Figure 5 in comparison with the experiments. Three theoretical calculations are presented; they show that two kinds of fluctuations contribute significantly to the lateral head group repulsions. First, the N^+ position fluctuates along the z axis normal to the bilayer (compare filled and open circles). The occasional fluctuations deep into the hydrocarbon phase cause very large lateral repulsions; in contrast, the fluctuations into the water phase contribute negligibly. Second, the rms width of the oil/water interface also affects the lateral interactions. B_2 increases significantly with the small change from $\Delta z = 3.0$ to 3.15 Å (compare filled circles and open squares). A broadened interface leads to a decreased gradient of the electrostatic free energy, with the consequence that the N^+ tends more toward the oil and the dipolar repulsions are higher.

An alternate representation of these interfacial fluctuations is as an increased "effective" dielectric constant of the heptane

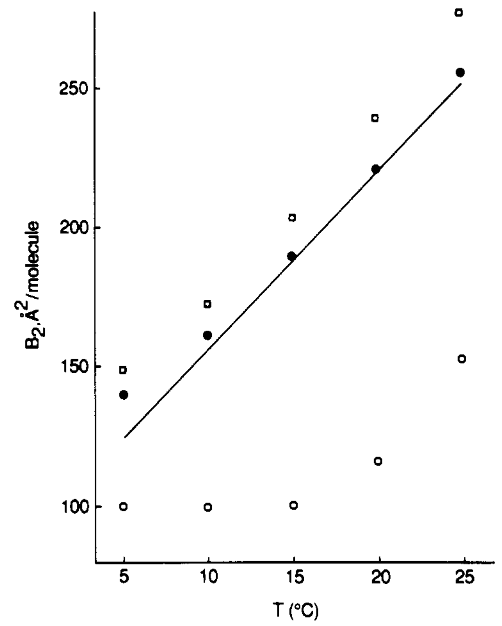

FIGURE 5: Second virial coefficient B_2 of lateral pressure/surface density isotherms of PC monolayers at heptane/water interface as a function of temperature. Solid line by Stigter and Dill (1988) from experiments by Taylor et al. (1976). Points from theory with eq 12 and 15: (●, ○) with $\Delta z = 3.00$ Å in eq 12; (□) with $\Delta z = 3.15$ Å in eq 12; (●, □) from eq 15 with fluctuating positions z_1 and z_2 of N^+ charges; (○) from eq 15 with N^+ charges fixed at average positions, $z_1 = z_2 = z = z_{av}$, with z_{av} from eq 13.

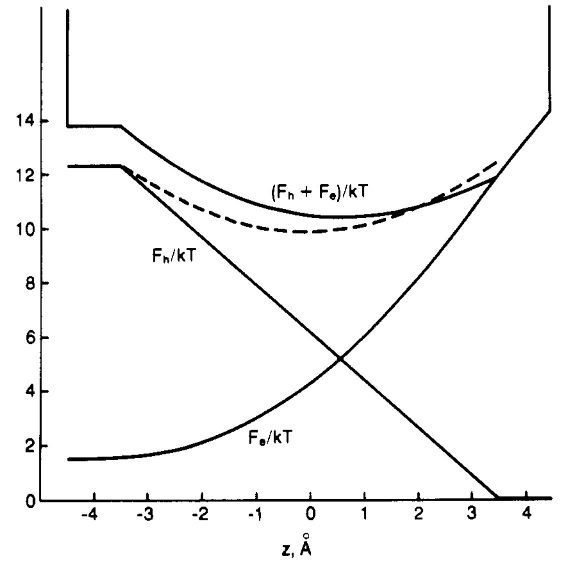

FIGURE 6: Free energy functions of PC near heptane/water interface. Electrostatic free energy F_e without interfacial fluctuations, $\Delta z = 0$ in eq 12; $\epsilon_h = 1.90$ times bulk value of heptane in eq 7–9. Remaining details as in Figure 4.

phase, ϵ_h. For example, if we set ϵ_h equal to 1.90 times its bulk value in eq 4–9, and $\Delta z = 0$ (no interfacial fluctuations) in eq 12, then we get the free energy profiles shown in Figure 6. The corresponding B_2 values are plotted in Figure 7 as circles from 5 to 25 °C and compared with the solid line for the experimental results as in Figure 5. There is not much difference between the $F_t(z)$ curves in Figures 4 and 6; in Figure 7 the agreement with experiments is nearly as good as in Figure 5 for $\Delta z = 3.00$ Å. This shows that a decrease of Δz may be represented alternatively as an increase of ϵ_h.

The agreement between theory and experiment in Figures 5 and 7 suggests that the present model captures essential aspects of head group behavior. Additional computations have shown the effect of some refinements of the model. In general,

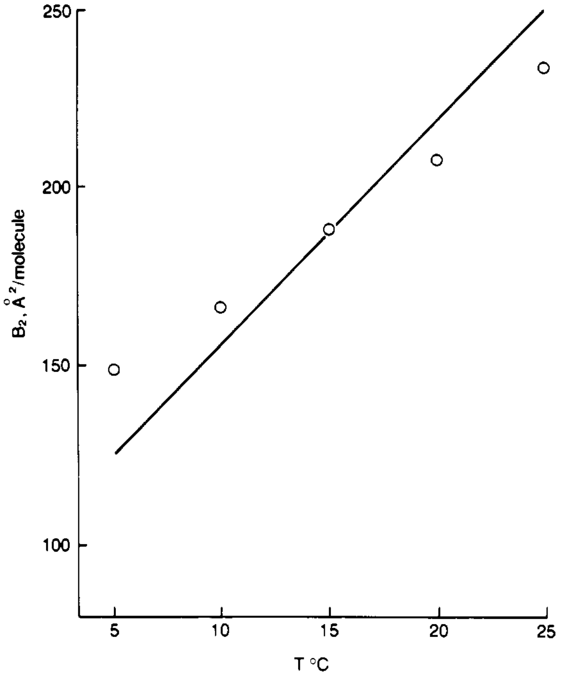

FIGURE 7: Second virial coefficient B_2 of PC at heptane/water interface as a function of temperature. Solid line from experiments as in Figure 5. Points from eq 15 with F_t curves as in Figure 6.

the position of the PC head group near the oil/water interface is a relatively robust prediction of the theory whereas the temperature dependence of B_2 is much more delicate. The change with temperature of the N^+ position depends not only on a shift of the $F_t - z$ curve but also strongly on its curvature, d^2F_t/dz^2. This curvature depends on details in the most uncertain parts of the F_h and F_e curves, just in the crossing region. Problems in determining F_e have been noted above. The macroscopic model of a sphere crossing a planar interface is crude also for F_h. The curvature of the $F_h - z$ curve is influenced by deviations from spherical shape or structural fluctuations of the N^+ charge and its hydrophobic shielding, and by a possible change of the oil/water interfacial area linked to the motion of the N^+ charge.

The use of the bulk dielectric constants of heptane and water in the above treatment needs some comment. The coefficient B_2 occurs in the virial expansion of the lateral pressure around head group density $\Gamma = 0$. So the potentials U_{12} and F_t in eq 15 for B_2 refer to two head groups at a clean interface, at $\Gamma = 0$. Possible changes of ϵ_w and ϵ_h near the interface, including those due to the mutual solubility of water and heptane, are expected to be insignificant; the interfacial fluctuations Δz, relevant to $F_e(z)$, are those of the clean interface. Strictly, any conclusion from B_2 concerning the head group dipole refers to the situation at $\Gamma = 0$.

PHOSPHATIDYLETHANOLAMINE AND OTHER PC ANALOGUES

Whereas in PC head groups the N^+ end is bonded to three methyls, in PE head groups the N^+ end is bonded to three hydrogens; there is no other structural difference. The X-ray diffraction (Hitchcock et al., 1974), neutron diffraction (Büldt & Seelig, 1980), and NMR evidence (Seelig & Gally, 1976) shows that the PE head group lies approximately parallel to the bilayer plane, as it does for PC. Thus it has been considered puzzling that the physical properties of the two types of phospholipids are so different (Hauser et al., 1981); those differences are often attributed to the possibilities for hydrogen bonding of PE to water, which are nonexistent for PC.

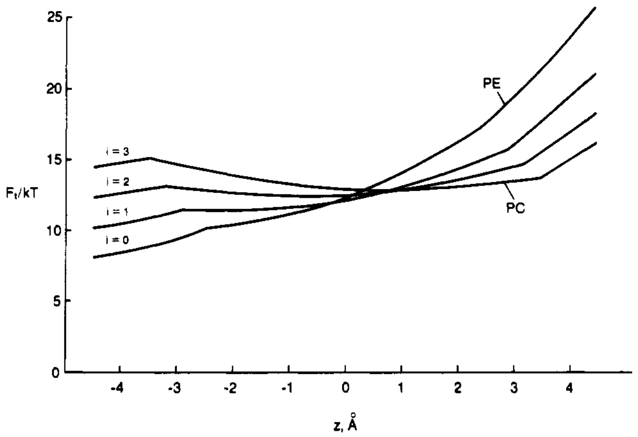

FIGURE 8: Total free energy curves $F_t(z)$ at 25 °C for PC, as in Figure 4, and for PC analogues with fewer (i) methyl groups attached to N^+.

Structural differences in molecular organization of head group orientation appear to be sufficiently small as to be within the experimental limits of error. Neutron diffraction of PE (Büldt & Seelig, 1980) has established the relative position of the methylene fragments of the head groups but cannot detect a trans to gauche change of α_5, which may shift N^+ by several angstroms. ^1H and ^{13}C high-resolution NMR of PC (Hauser et al., 1980) indicates a gauche conformation of α_5 under various conditions; the temperature dependence has not been studied. The overall head group orientation may not be constant: at a fixed conformation a change of only 6° in the angle between the P–O bond in the PN link and the bilayer plane corresponds to a normal displacement of N^+ by about 0.5 Å.

Here we suggest that a principal difference is that the ethanolamine group is much less hydrophobic than the choline group, leading to a significant difference in head group orientation, particularly at higher temperatures.

We have made calculations for the series of analogues $-PO_4^--C_2H_4-N^+-(CH_3)_iH_{3-i}$, where $i = 0$ for PE and $i = 3$ for PC, assuming that in eq 3 $n_H = 4 + 3i$ and in eq 2–12 $t = 2.6 + 0.3i$ Å and $\Delta z = 3$ Å. Results for F_t at 25 °C, presented in Figure 8, show the effect of successive removal of one methyl group at a time from PC. The breaks in the curves occur when the hydrophobic sphere leaves the interface, at $z = \pm t$. They would be smoothed out in an improved model in which the connecting $-C_2H_4-$ group is not considered part of the charged sphere but is placed between the dipole charges on the PN axis. The series has progressively less tendency for the N^+ end of the head group to be embedded in the hydrocarbon phase. Going from PC to PE at 25 °C, the model predicts for the average position of the N^+ charge $z_{av} = 0.50$, -1.14, -2.64, and -3.47 Å, respectively; the corresponding rms fluctuations of N^+ are $\mu = 1.89$, 1.95, 1.57, and 0.97 Å. The latter results are consistent with the rms fluctuations of the distal CD_2 fragment in the head group measured by neutron diffraction: $\mu = 2.1 \pm 0.4$ Å at 20 °C and 2.5 ± 0.4 Å at 70 °C for PC (Büldt et al., 1979), and $\mu = 0.9$ Å at 25 °C for PE (Büldt & Seelig, 1980).

The above results for z_{av} correspond to an average out-of-plane angle $\theta = \arcsin(z_{av}/l)$ of the PN vector into the hydrocarbon phase (see Figure 2), of $\theta = 6°$ for PC and $\theta = -50°$ for PE. Within the experimental uncertainties, these results are not inconsistent with neutron diffraction experiments from which Büldt et al. conclude that the PC head group is nearly parallel with the bilayer (Büldt et al., 1979) and that the PE head group is oriented by a small angle out of the plane of the bilayer into the water (Büldt & Seelig, 1980).

The model predicts that PE is oriented with the N^+ directed into the water phase, and this should change little with temperature. Because dipolar interactions in water are about 3 orders of magnitude weaker than in oil, they contribute little to the lateral pressure among PE molecules. Therefore, lateral repulstions among PE head groups are predicted to be largely due to steric interactions. This prediction is confirmed by unpublished experiments of the lateral pressure vs area of PE monolayers at the heptane/water interface, kindly made available to us by Dr. J. Mingins. Analysis of two sets of data yields $B_2 = 139$ and 129 Å^2/molecule at 5 °C and $B_2 = 124$ and 132 Å^2/molecule at 20 °C. These values are not much higher than expected from a hard disk model of the PE head group. The small difference might well arise from rotation of the head group in the interface, see Figure 1; in dilute monolayers the two alkyl chains of the lipid are not necessarily aligned above the head group, as assumed in the hard disk model. Furthermore, repulsion due to the ester dipoles in the phospholipids, see Figure 1, may make a small contribution to B_2 which we have neglected in the calculations. This explains why at 5 °C the B_2 values, and hence the repulsions, are virtually the same for PE and PC; their interactions diverge only at increasing temperature.

Values of B_3/B_2^2, derived from the PE data supplied by Dr. Mingins, are 0.628 and 0.864 at 5 °C and 0.923 and 0.782 at 20 °C, in good agreement with the hard disk value 0.782 (Ree & Hoover, 1964). On the other hand, from the experimental data for PC at 5 °C we found (Stigter & Dill, 1988) the higher value $B_3/B_2^2 = 1.39$. The difference might be correlated with the larger values of z_{av} and μ for PC which perhaps influence B_3 more than B_2.

In the PE to PC series the gradual transfer of N^+ from water into hydrocarbon correlates well with the critical micelle concentration (cmc) of dodecyl sulfate with various counterions in water at 25 °C (Mukerjee & Mysels, 1970). In the series $C_{12}SO_4NH_4$, $C_{12}SO_4NH_3CH_3$, and $C_{12}SO_4N(CH_3)_4$ the cmc's are 6.16×10^{-3}, 5.70×10^{-3}, and 5.52×10^{-3} M, respectively. The decrease of the cmc in the series indicates more favorable formation of micelles, presumably due to increased shielding of the repulsion among SO_4^- groups in the micelle surface. This is observed in spite of the increasing size of the counterions in the series, which makes electrostatic shielding more difficult. Hence, the decrease of cmc can only be explained by a specific adsorption of counterions at the micelle surface, between the head groups at the water/hydrocarbon interface, which increases significantly from NH_4^+ to $N^+(CH_3)_4$. A very similar increase of adsorption of N^+ to hydrocarbon is observed in the PE to PC series.

Because of the reduced symmetry relative to PC or PE, there may be other subtle contributions to head group orientation, not taken into account here for the mono- and dimethyl-substituted PE compounds, $i = 1$ and 2. The model of the hydrophobic sphere around N^+ implies free rotation around the H_2C-N bond; see Figure 1. This might be a good approximation for PC and PE, but the methyl groups in $N(CH_3)H_2$ and $N(CH_3)_2H$ for the less symmetrical intermediate compounds will have a definite preference for the oil phase, thus restricting rotation around the H_2C-N bond when N is near the interface. Therefore, these analogues may be more similar to PC than suggested by Figure 8.

The present model also does not take into account possible hydrogen bonding of the head group of PE or of the mono- or dimethylamine to water. Hydrogen bonding would contribute, as does the reduction of hydrophobicity, to the inclination of the N^+ end of the head group to favor the water phase relative to the hydrocarbon phase. However, inasmuch as the N^+ end of the PE head group already favors the water, due only to the balance of electrostatic and hydrophobic (CH_2) propensities, then hydrogen bonding should have little additional effect.

We have also made calculations of the CH order parameters in the PC head group, determined by NMR (Seelig & Seelig, 1980). To this end we have carried out conformational energy computations of the PC head group as a function of the dihedral angles α_3, α_4, and α_5 (see Figure 1), using the program AMBER (Weiner & Kollman, 1981; Weiner et al., 1984), which was provided generously by Prof. Peter Kollman. We found several low-energy conformations, less than a few kT apart, but with widely different sets of order parameters. Hence, in contrast to suggestions in the literature (Seelig et al., 1977; Skarjune & Oldfield, 1979), it is likely that the theoretical prediction of the order parameters requires consideration of conformations at more than one energy level. Unfortunately, in these molecular mechanical simulations the conformational energies depended significantly on the assumed value of the dielectric constant; the results, therefore, were much too uncertain to make meaningful predictions of order parameters.

APPLICATIONS TO MEMBRANE PROPERTIES

It is interesting to extrapolate results from the dipole model for B_2, derived at head group density $\Gamma = 0$, to the higher densities encountered in bilayer membranes. The important issue is then the dependence on Γ of the orientation of the head group dipoles. That this dependence on Γ might well be negligibly small is supported by measurements of the third virial coefficient, B_3. A significant dependence of head group orientation, and hence of B_2, on Γ should manifest itself as a contribution to B_3. The comparison of experimental B_3 values for PC with calculations of B_3 for stationary dipoles suggests no large changes of head group orientation with Γ (Stigter & Dill, 1988). Calculations of B_3 for fluctuating dipoles have not yet been made.

When the monolayer results are extrapolated to higher surface densities, the weaker lateral repulsions among PE head groups compared with PC head groups are likely to be responsible for some differences in bilayer properties. For example, the melting temperatures are about 20–30 °C higher for PE bilayers than for PC bilayers (Szoka & Papahadjopoulos, 1980). The weaker lateral repulsions permit PE head groups to pack more closely than PC, so they can more readily form phases of reversed curvature (Gruner, 1985) such as the inverted cylinders in the hexagonal H_{II} phase (Ellens et al., 1986a,b; Gagne et al., 1985; Caffrey, 1985). For the same reason, they should be favored in environments of higher surface density. Thus PE is relatively concentrated on the inside monolayers of bilayer membranes in bacteriophage PM2 and the bacterium *Bacillus megaterium* (Rothman & Lenard, 1977) and of small vesicles of egg phospholipids (Berden et al., 1975; Litman, 1974) and of synthetic mixtures of dimyristoyl-PC/dimyristoyl-PE (Lentz & Litman, 1978). As mentioned before, the difference in behavior between PE and PC is very temperature dependent and vanishes around 5 °C. This might cause subtle, but perhaps useful, differences, e.g., in the stability of mixed PE/PC vesicles (prepared) at 5 °C vs those at higher temperatures.

The head group interactions may also be correlated with the equilibrium separation d between bilayers in water. (i) The direct force measurements by Marra and Israelachvili (1985) at 21 °C yielded d = 20–25 Å between dipalmitoylphosphatidylcholine (DPPC) and d = 12 Å between dipalmitoylphosphatidylethanolamine (DPPE) bilayers. (ii) Rand (1981) reports that, from osmotic compression of bilayer suspensions in water by an external dextran solution, one finds that for DPPC the bilayer separation depends on temperature, increasing from d = 19.6 Å at 25 °C to d = 32.8 Å at 50 °C.

The above experiments suggest that the repulsion between DPPC bilayers is greater than that between DPPE bilayers and increases with temperature, just as does the lateral repulsion between the head groups. The bilayer interaction theory, however, is not so simple. The contribution of the head group dipoles to the total bilayer interaction is zero to first order, because smeared dipolar layers do not interact. Higher order calculations depend strongly on model assumptions (Jönsson & Wennerström, 1983, 1985), and convincing predictions have not yet been made. Our head group model will make such predictions more realistic in two ways. First, the normal dipole fluctuations can now be linked to the potential field for head group tilt, in agreement with head group fluctuations from neutron scattering experiments. Second, the calculated bilayer interaction depends strongly on the assumed lateral dipole distribution in each layer. This lateral distribution can be chosen to yield the correct lateral pressure, extrapolated from monolayer data up to near-bilayer head group densities (Taylor et al., 1976).

The contribution of the head group dipoles to the potentials across monolayers and bilayers (Hladky, 1974; Mingins, 1987), and to the binding and translocation of ions in bilayers (Lieberman & Topaly, 1969; LeBlanc, 1969; Haydon, 1975; Haydon & Hladky, 1972; Flewelling & Hubbell, 1986a,b), is not clear. The incomplete experimental data show that differences between PE and PC are not large, suggesting that other factors such as water dipoles are of major importance. A more complete experimental comparison between PE and PC bilayers would be of obvious interest.

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APPENDIX

The treatment of interfacial fluctuations has been developed, in particular by Mandelstam (1913), as part of the theory of light scattering by liquid interfaces and, more recently, has been applied to liquid films by Vrij (1964). We consider a square interfacial area between oil and water, bounded by $x = \pm a/2$ and $y = \pm a/2$. The fluctuations or corrugations of the interface are expressed in a Fourier series of the perpendicular coordinate z:

$$z = \sum_{\rho=-n}^{+n} \sum_{\sigma=-n}^{+n} \zeta_{\rho,\sigma} e^{2\pi i(\rho x+\sigma y)/a} \qquad \text{(A1)}$$

The pair relations between the complex Fourier coefficients $\zeta_{\rho,\sigma} = \zeta^*_{-\rho,-\sigma}$ ensure that z is real; that is, eq A1 represents a set of two-dimensional vibrations, or capillary waves, with wavelengths $a/(\rho^2 + \sigma^2)^{1/2}$.

When a corrugation expands the interfacial area by ΔA, and γ is the interfacial tension, the work associated with the corrugation is

$$W = \gamma \Delta A \qquad \text{(A2)}$$

Since for small amplitudes

$$\Delta A = \frac{1}{2}\int_{-a/2}^{a/2}\int_{-a/2}^{a/2}\left[\left(\frac{\mathrm{d}z}{\mathrm{d}x}\right)^2 + \left(\frac{\mathrm{d}z}{\mathrm{d}y}\right)^2\right]\mathrm{d}x\,\mathrm{d}y \qquad \text{(A3)}$$

eqs A1 and A2 yield

$$W = 2\pi^2\gamma\sum_{-n}^{n}\sum_{-n}^{n}|\zeta_{\rho,\sigma}|^2(\rho^2 + \sigma^2) \qquad \text{(A4)}$$

According to the equipartition theorem (Einstein, 1910) the mean value of each term in the series for W is $kT/2$, giving a mean-square amplitude of each Fourier term

$$|\zeta_{\rho,\sigma}|^2 = \frac{kT}{4\pi^2\gamma(\rho^2 + \sigma^2)} \qquad \text{(A5)}$$

Summing over all Fourier terms, eq A5 gives for the mean-square fluctuation of the interface

$$\Delta z^2 = \frac{kT}{4\pi^2\gamma}\sum_{-n}^{n}\sum_{-n}^{n}\frac{1}{\rho^2 + \sigma^2} \qquad \text{(A6)}$$

In eq A1 the term with $\rho = \sigma = 0$ gives the equilibrium value for z, excluded from the summation in eq A6.

The vibrations of interest have a wavelength of the order of molecular size or longer, with the upper limit corresponding to the effective range of the electrostatic field in F_e, limiting the sums in eq A6 to perhaps $n = 100$ or 1000. It appears that Δz is not very sensitive to the value chosen for n, the increase with n being logarithmic for large n. With $kT/\gamma \cong 8.0$ Å^2 for the oil/water interface at 25 °C, eq A6 gives for $n = 30$, 100, 300, and 1000 the values $\Delta z = 2.24$, 2.56, 2.82, and 3.08 Å, respectively. We expect that in the treatment of F_e the effective fluctuations of the oil/water interface are around 2.5 or 3 Å, in line with recent experiments on the interfacial reflectivity, measured by low-angle X-ray scattering (Robinson, 1987; Weiss et al., 1987).

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